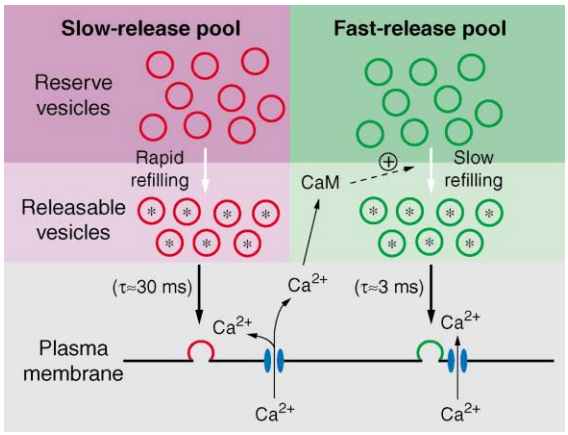

The Dynamics of Depletion and Refilling of Vesicle Pools at the Calyx of Held in Response to Ca^{2+} Influx through Voltage-Gated Ca^{2+} Channels

Asterisks indicate vesicles that are primed for Ca^{2+}-triggered release. CaM = calmodulin.

demonstrating a selective effect of Ca^{2+} on the replenishment of one kinetic component of release—the fast-release pool—and by proposing a role for calmodulin in the linkage between Ca^{2+} and the rate of pool refilling. Because the process by which reserve vesicles become releasable remains largely mysterious, the molecular mechanism underlying the acceleration of replenishment by Ca^{2+}/calmodulin cannot as yet be specified. Also, calmodulin has many molecular targets, and so the possibility exists that Ca^{2+}/calmodulin might influence replenishment indirectly by altering other cellular processes that in turn affect the priming or availability of vesicles.

What is the physical manifestation of the two functionally defined pools of releasable vesicles described by Sakaba and Neher? One possibility is that the pools are intermingled at each active zone, with the fast- and slow-release vesicles differing in biochemical state or in proximity to Ca^{2+} channels. Alternatively, the two pools may arise from distinct active zones in the presynaptic terminal, with each active zone having either high release probability but slow recovery, or low release probability but fast recovery. If the latter scenario holds, it is possible that the two types of active zones represent different stages of developmental maturity. At the ages studied by Sakaba and Neher (2001), synaptic transmission at the calyx of Held has not yet reached its mature form. As the synapse matures, it switches from rapid to slow depression and becomes capable of reliably supporting high-frequency transmission (Taschenberger and von Gersdorff, 2000; Iwasaki and Takahashi, 2001). This ability to follow high presynaptic frequencies without depressing is accompanied by a decrease in release probability. Although changes in action potential waveform may in part account for decreased release probability during maturation, alterations in the inherent release characteristics of the active zone may also contribute. A brief depolarization, such as an action potential, would release relatively little of Sakaba and Neher's slow-release pool, making it resistant to synaptic depression during trains of action potentials. By contrast, the fast-release pool would deplete rapidly. Thus, the fast-release pool has characteristics similar to immature, rapidly depressing synapses, whereas the slow-release pool is similar to mature, depression-resistant synapses at the calyx of Held. However, because the experiments with more mature synapses were done without presynaptic voltage control, it is not yet clear whether this suggestion for the basis of maturation is reasonable. Maturation of the synapse could alternatively reflect increased activity of Sakaba and Neher's calmodulin-dependent pathway, which accelerates refilling of the fast-release pool. Enhanced refilling would also make the mature synapse more resistant to depression.

Although the focus of Sakaba and Neher's work is the depletion and replenishment of releasable vesicles, we should keep in mind that recovery from depletion of releasable pools of vesicles is not the only short-term process modulating synaptic efficacy. Calcium-dependent facilitation also affects release on the same time scale (Wu and Borst, 1999), as do postsynaptic receptor desensitization and saturation (Neher and Sakaba, 2001; Sun and Wu, 2001). It remains for future work to establish how these and other processes act in combination with vesicle depletion and pool refilling to produce the full range of slowly subsiding aftereffects envisioned by Katz.

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Protein Phosphatase 1 and LTD: Synapses Are the Architects of Depression

NMDAR-dependent long-term depression involves the activation of protein phosphatase 1 (PP1) and 2B (calcineurin) and the subsequent dephosphorylation of synaptic proteins. In this issue of *Neuron,* Morishita et al. (2001) provide evidence that precise targeting of PP1 to synaptic substrates is critical for the expression of LTD.

Although controversy rages about the expression mechanisms of hippocampal long-term potentiation (LTP), a relatively simple and consistent story exists to explain its counterpart, NMDAR-dependent homosynaptic long-term depression (LTD). This form of LTD, one of two types of synaptically induced LTD of naive inputs in the hippocampus, was the first to be described (Mulkey and Malenka, 1992; Dudek and Bear, 1992). Early mechanistic studies provided compelling evidence that LTD involves the activation of two protein phosphatases found at synapses, protein phosphatase 2B (PP2B, also known as calcineurin) and protein phosphatase 1 (PP1; Mulkey et al., 1993). Subsequent studies demonstrated that the GluR1 AMPAR subunit was dephosphorylated during LTD (Lee et al., 1998, 2000), suggesting that the AMPAR itself is the target for phosphatases during LTD. However, it is unclear how activated protein phosphatases are able to dephosphorylate specific protein substrates among the myriad of proteins present at synapses. Morishita et al., 2001 (this issue of *Neuron*) provide some important new clues as to how this is achieved by PP1.

In this study, the authors investigate the effects of blocking the binding of interacting proteins containing a common RKIXF motif binding site to PP1. Of the identified proteins that bind PP1, inhibitor-1 (I-1), neurabin I, spinophilin (also known as neurabin II), yotiao, and NF-L are of particular relevance here because they are found at excitatory synapses. Indeed, spinophilin has been shown to be essential for LTD, since spinophilin knockouts do not express LTD (Feng et al., 2000). Morishita et al. used three short peptides to investigate the functional effects of blocking PP1 protein interactions by loading CA1 pyramidal neurons with peptide during whole-cell patch-clamp recordings from hippocampal slices. Two of these peptides were sequences from the PP1 binding proteins, Gm and I-1, which contained the RKIXF binding motif and blocked the binding of proteins to PP1. A third inactive control peptide was derived from a mutation of I-1 and lacked the full PP1 binding motif. The active peptides but not the control peptide blocked NMDAR-dependent LTD in patch-clamped cells, while neighboring cells still expressed robust LTD. Importantly, none of the peptides had any effect on NMDAR-mediated EPSCs, allowing us to conclude that protein-protein interactions involving PP1 are involved in LTD downstream of induction via NMDAR activation.

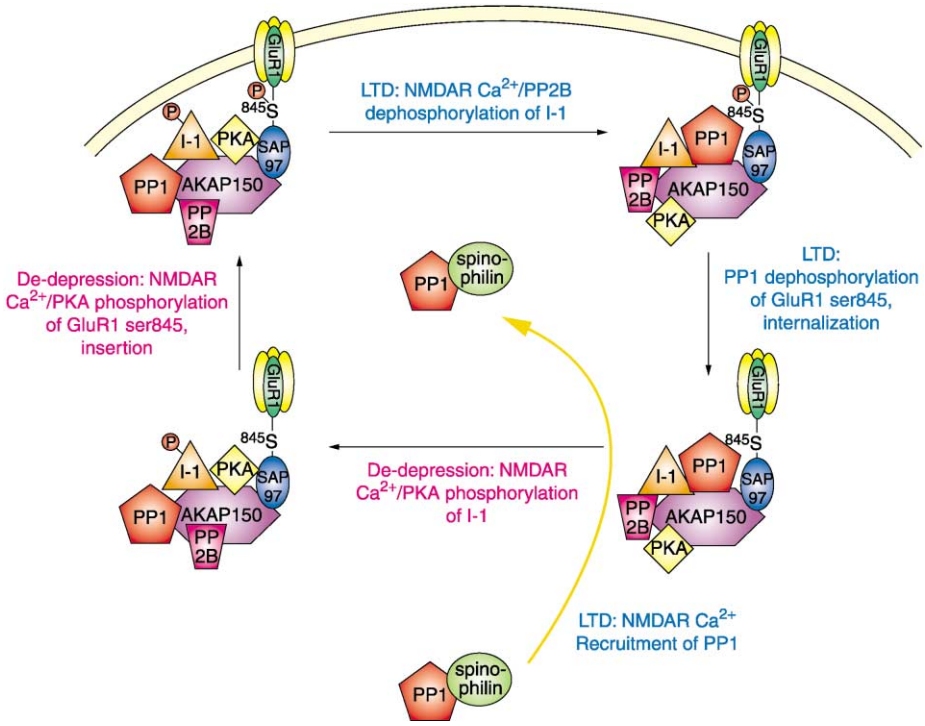
Furthermore, this role of PP1 binding proteins is specific for NMDAR-dependent LTD, since Gm and I-1 peptides did not block mGluR-dependent LTD or chemLTD, induced by bath application of DHPG and NMDA, respectively. mGluR-dependent LTD has been previously shown to have different mechanisms from NMDAR-dependent LTD; the lack of effect of the active peptides further supports the idea that these two forms are mechanistically distinct. The lack of effect on chemLTD is consistent with the original finding that this form of LTD is not dependent upon PP1. However, chemLTD and synaptically induced NMDAR-dependent LTD mutually occlude (Lee et al., 1998), making this differential requirement for PP1 rather puzzling.

An important insight into the tight and precise regulation of AMPAR-mediated synaptic transmission was provided by comparing the effects of the peptides on LTD with those on basal synaptic transmission. Previous studies have shown that blocking PP1 interactions with similar peptides causes a run-down in AMPAR-mediated responses to exogenously applied agonist (e.g., Yan et al., 1999). In the present study, however, Gm and I-1 had no effect on synaptic AMPARs, since baseline EPSC amplitude was unaffected. Moreover, loading cells with a constitutively active form of PP1 had no effect on basal transmission. These results are rather surprising, since one might expect that loading excess activated PP1 into cells would bypass the need for NMDAR activation and directly cause a depression of transmission. In an elegant experiment, Morishita et al. monitored the effects of loading with PP1 on nonsynaptic AMPARs by recording the response to exogenously applied kainate while simultaneously monitoring EPSCs. Unlike synaptic AMPARs, nonsynaptic AMPARs were indeed susceptible to regulation by PP1; it seems that synaptic AMPARs need another ingredient to allow such regulation. The data in the present study also serve as a cautionary tale for studies of glutamate receptor function that measure effects on nonsynaptic receptors or recombinant receptors in nonneuronal cells. Synaptic receptors appear to be under much tighter control than nonsynaptic receptors, presumably because of the presence of a host of regulatory interacting proteins that are specifically located at synapses.

What is the extra ingredient necessary to enable PP1 to regulate synaptic AMPARs? Loading with PP1 only had an effect when NMDARs were activated during an LTD-induction protocol. PP1 had no extra effect on LTD induced using a standard strong induction protocol; however, it greatly facilitated LTD when a weak induction protocol was used that on its own produced almost no depression. Thus, the extra ingredient to allow PP1 to regulate synaptic AMPARs appears to be Ca^{2+} influx through synaptic NMDARs.

The authors propose a model in which NMDAR activation causes a targeting to, or enables access of, PP1 to synaptic substrates (possibly AMPARs) that then allows dephosphorylation and a reduction in synaptic strength. One prediction from this, therefore, is that prior induction of LTD should render synaptic AMPARs in a state in which they are under direct modulation by active targeted PP1; thus, inhibition of PP1 targeting by either the Gm or I-1 peptides should reverse previously established LTD. Indeed, in a difficult set of two-pathway experiments, Morishita et al. show that only a pathway that had previously undergone LTD and not a control pathway exhibited an increase in EPSC amplitude with perfusion of the Gm or I-1 peptides. Thus, protein-protein interactions involving PP1 and binding proteins containing the RKIXF motif play a critical role in targeting PP1 to the relevant synaptic substrates during LTD.

When thinking about how this could be achieved, two models come to mind. PP1 binding proteins could be involved in the rapid recruitment of PP1 to synapses in response to NMDAR activation. Alternatively, PP1 may already be present at synapses but interaction with PP1 binding proteins and NMDAR activation leads to a rapid alteration in the architecture of synaptic protein complexes that then allows access of PP1 to the relevant substrates. To address this question of mechanism, Morishita et al. used immunocytochemical techniques in cultured neurons to compare the localization of PP1



A Model for the Role of PP1 Targeting in the Regulation of Synaptic AMPAR Function

with that of the presynaptic protein synaptophysin. While only a subset of synapses contained PP1 in the absence of LTD, in neurons that had undergone LTD induction there was a far greater colocalization, suggesting a rapid, NMDAR-dependent redistribution of PP1 from the dendrites to synapses. There was also an increase in dendritic PP1 signal, presumably reflecting movement of protein from the soma out into the dendrites. However, it is unclear whether this recruitment is rapid enough to mediate the initial phase of LTD, since the immunocytochemical assay was only performed at a single time point, 10 min after LTD induction. Furthermore, although the recruitment of PP1 to synapses is associated with LTD in culture, there is as yet insufficient evidence as to whether it is responsible for mediating the depression; e.g., does the observed increase in PP1 colocalization with synaptophysin correlate with the magnitude of depression measured in culture?

There is an ever-growing list of proteins that bind directly to, or are associated with, AMPARs at synapses (Sheng and Lee, 2001). Much of this work has focused on the GluR2 subunit; however, it is now becoming clear that a highly organized molecular complex is also associated with the GluR1 subunit (Fraser and Scott, 1999), which is involved in the highly specific differential regulation of AMPAR function during LTP and LTD. In addition, the present study suggests that components of this complex may also be rapidly recruited to synapses during plasticity. One model to bring together recent findings with the results from Morishita et al. is shown in the Figure. PP1 is targeted to the complex associated with GluR1 that includes SAP97, AKAP150, PKA, and PP2B (Fraser and Scott, 1999). In this model, I-1 and PP1 are hypothetically proposed also to be associated with the complex. During LTD, PP2B dephosphorylates I-1, which disinhibits targeted PP1. This induces a change in the architecture of the complex, allowing PP1 to maintain a tonic dephosphorylation of serine 845 on GluR1 that leads to the internalization of AMPARs. In addition, during LTD, spinophilin (or another PP1 binding protein) causes translocation of PP1 from the dendrites to synapses, which may recruit PP1 to the complex at synapses that initially lack this component. During dedepression, there is a PKA-dependent phosphorylation of I-1. This inhibits PP1 and changes the organization of the complex so that PKA-dependent phosphorylation of serine 845 occurs, leading to reinsertion of the AMPAR complex. An additional possibility is that once reinserted, PP1 dissociates from the complex and translocates away from the synapse, thus making the rapid recruitment of PP1 to synapses an important requirement during LTD.

This is one possible model to explain the differential regulation of AMPAR function by targeted PP1 and PKA. It does not address the role of GluR2-associated proteins and the phosphorylation state of serine 880 on GluR2, which has recently been shown to be important for regulating AMPAR surface expression during LTD (e.g., Daw et al., 2000; Kim et al., 2001). In this regard, it would be of interest to know if PP1 dephosphorylates serine 880 on GluR2 during LTD. Indeed, it is not clear what proteins PP1 targets to cause the expression of LTD. Although it is assumed for the purposes of this model that serine 845 on GluR1 is a target, this has not as yet been demonstrated. This residue has been shown to be dephosphorylated during NMDAR-dependent LTD (Lee et al., 2000) and chemLTD (Lee et al., 1998); however, for chemLTD at least, this dephosphorylation is not

dependent upon PP1. Dephosphorylation also occurs at another site on GluR1, serine 831, during depotentiation, another form of depression (Lee et al., 2000). However, the identity of the phosphatase responsible for this dephosphorylation is also unknown. Another related issue is the unknown functional consequence of such dephosphorylation events. Studies on recombinant receptors indicate that dephosphorylation of serine 845 may decrease the number of receptors that open in response to glutamate, due either to a decrease in the probability of channel opening on binding glutamate or a reduction in the number of surface-expressed channels. Dephosphorylation of serine 831 is reported to decrease the mean channel conductance of recombinant GluR1 homomers. However, there have to date been no reports of the functional effects of dephosphorylation of these residues during LTD or depotentiation for synaptic AMPARs in neurons.

The marriage of cell biological techniques and functional studies of synaptic glutamate receptor function has in recent years provided increasing evidence that protein-protein interactions lead to a precise targeting of synaptic proteins that is critically important for the regulation of synaptic strength. Clearly, however, there is plenty more to do before a full understanding can be achieved of how the molecular architecture at excitatory synapses orchestrates the precise regulation of synaptic glutamate receptors during plasticity.

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Determinants of Spike Timing-Dependent Synaptic Plasticity

Recent studies show that the precise timing of presynaptic inputs and postsynaptic action potentials influences the strength and sign of synaptic plasticity. In this issue of *Neuron*, Sjöström and colleagues (2001) determine how this so-called spike timing-dependent plasticity depends on the frequency and strength of the presynaptic inputs.

One of the advantages of in vitro brain slice experiments over in vivo whole animal experiments (apart from the fact that you can get home in time for dinner) is that they allow precise control over the experimental conditions. The disadvantage is that in vitro experiments are done within an environment isolated from the natural activity of the network (but see Sanchez-Vives and McCormick, 2000). The challenge for the "brain slicers" is therefore to relate the phenomena they see in vitro to what happens in vivo. This issue is particularly relevant to recent work showing that the precise millisecond timing of presynaptic inputs and postsynaptic action potentials has a powerful influence over the expression and sign of synaptic plasticity.

Recent studies have shown that repetitive activation of excitatory postsynaptic potentials (EPSPs) within a brief time window before action potentials (APs) causes long-term potentiation (LTP), whereas EPSP activation just after APs leads to long-term depression (LTD) of synaptic transmission (see Figure, panel A; Linden, 1999). On the face of it, this so-called spike timing-dependent plasticity (STDP) would appear to provide a simple learning rule, which could in principle underlie memory formation. As usual, though, things are never that simple. In vivo, neurons are continuously bombarded with ever-changing patterns of synaptic input, resulting in highly irregular patterns of AP output. How does the almost "random" nature of synaptic input and output in vivo influence the timing relationships for the induction of synaptic plasticity? The paper by Sjöström and colleagues (Sjöström et al., 2001) in the current issue of *Neuron* addresses this question using paired recordings in brain slices of rat visual cortex. By determining the dependence of STDP on the rate, timing, and strength of presynaptic inputs, they come up with a model that can predict the sign and strength of STDP during random pairings at different frequencies. Along the way, they also come up with some interesting surprises.

One of the fundamental requirements for LTP induction is thought to be cooperativity, whereby a weak input will only undergo LTP if activated together with a strong input (Bliss and Collingridge, 1993). To overcome this requirement during activation of single presynaptic inputs, AP firing in response to somatic current injection is commonly used to simulate the strong input. According to the standard STDP timing curve (Figure, panel A), repetitive activation of EPSPs just before APs should lead to LTP. It turns out that this is not always the case. Sjöström and colleagues find that whether a weak input undergoes LTP when paired with somatic APs depends